WILDFIRE, STREAM CHANNEL DISTURBANCE, AND GENETIC VARIATION WITHIN AQUATIC VERTEBRATE POPULATIONS

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Helen Neville
University of Nevada-Reno
Department of Biology

Amanda Rosenberger
University of Idaho
Ecohydraulics Research Group

Jason Dunham
USGS FRESC Corvallis Research Group
(Forest and Rangeland Ecosystem Science Center)

INTRODUCTION

Concern over the potential risks posed by wildfire and wildfire management practices in the western United States has become increasingly focused on aquatic ecosystems (e.g.,Rieman and Clayton 1997; Dunham et al. 2003; Pilliod et al. 2003; Rieman et al. 2003; Burton 2005). In particular, wildfire management is strongly influenced by regulations that protect the growing number of species that are threatened, endangered, or of special concern. Among these, native trout are an important focus, and consequently there is a need to develop more useful information for evaluating the effects of wildfire on various aspects of trout ecology, evolution, and persistence.

Disturbance to stream channels following a burn is likely one major effect of wildfire on trout populations. The reorganization of channels and disturbance caused by landslides, floods and debris flows (Benda et al. 2003; Miller et al. 2003; Wondzell and King 2003) can cause massive mortality of trout (Rieman et al. 1997; Burton 2005), leading to demographic bottlenecks and even the extirpation of local populations immediately following such an event (Dunham et al. 2003). Additionally, the loss of vegetation caused by wildfires may result in increased stream temperatures that decrease the extent of thermally suitable habitat, potentially affecting both the distribution of a species and the size of persisting populations over a longer time-frame (Pilliod et al. 2003; Bury 2004; Burton 2005). Trout possess several characteristics, such as strong dispersal abilities and variable migratory life histories, which may help them cope with the highly dynamic landscape presented naturally by fire (Dunham et al. 2003). However, the ability of trout species to recover from fire-related habitat alterations likely depends on

landscape context, and may be increasingly hindered as human influences such as roads and barriers become more prevalent (Rieman *et al.* 2003).

Information on the effects of wildfire on native trout is still limited (Rieman *et al.* 2003), partly due to the difficulty of tracking demographic responses to fire across a landscape and over long time-frames. Genetic data may provide useful insight in such cases, as population bottlenecks or extinction-colonization events due to fire would be expected to impact genetic patterns within populations. Population turnover or severe bottlenecking is generally thought to erode genetic variation (Gilpin 1991; Harrison and Hastings 1996; Hedrick and Gilpin 1997; Hedrick 2001), and in several instances genetic variation has been used to assess the effects of disturbance (Curtis and Taylor 2004; Baucom *et al.* 2005; Wahbe *et al.* 2005). As evidence is mounting that genetic diversity can have important ramifications for populations in terms of resistance to future disturbance (Hughes and Stachowicz 2004) and extinction risk (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Madsen *et al.* 1999; Frankham 2005), improving our understanding of the genetic responses of native trout to disturbances such as fire is becoming increasingly important for the conservation of natural populations. Studies where fire disturbance histories and interactive landscape factors are well understood may be particularly valuable.

Our objectives in this study were to examine the impact of disturbance and habitat fragmentation on genetic diversity in native rainbow trout (*Oncorhynchus clarkii mykiss*) populations persisting in a large watershed in central Idaho with broad-scale historical

disturbance due to fire. Fire is a natural and common component of this high-desert ecosystem (Burton 2005) that over the past several decades has generated disturbances of varying intensities ranging from simple loss of riparian vegetation with little long-term impact to aquatic species to complete channel reorganization and the assumed extirpation of resident species from floods or debris flows following fire (see, e.g., Dwire and Kauffman 2003; Kiffney *et al.* 2004). In many respects this is an ideal study system for addressing the genetic effects of disturbance, as both disturbance histories and other interacting human effects are well-documented. In addition to fire, habitat fragmentation due to culvert barriers is one human impact that may be expected to affect trout populations strongly, both demographically and genetically (Koizumi and Maekawa 2004; Wofford *et al.* 2005; Neville *et al.* In press-a). In this study, therefore, we categorized the level of disturbance in each of fifty-five streams as 'undisturbed', 'burned', and 'burned and reorganized' (see below), and the level of fragmentation as 'connected' or 'isolated' based on the presence or absence of culverts. We estimated several metrics of genetic diversity to assess the influence of past demographic bottlenecks in this system on genetic variation. We hypothesized that genetic diversity would be negatively correlated with the intensity of disturbance (*i.e*, highest in 'undisturbed' streams, lowest in 'burned and reorganized streams'). Secondly, we sought to evaluate the relative importance of alternative impacts (natural fire *versus* human-placed culverts) on genetic diversity.

METHODS

Study site and field sampling procedures

This study took place in the Boise and Payette river basins in central Idaho, an ideal region for studying wildfire-related disturbance as significant portions of these basins burned between 1992 and 2003. We confined our selection of streams to those with perennial flow along a range of elevations (973-2096 m) likely to support rainbow trout, selecting headwater streams for sampling because they are most likely to show long-term effects of fire and fire-related disturbance. In addition, because non-native cutthroat trout have been introduced throughout the last century in the Boise and Payette river basins, we chose streams that were far from reservoirs and lakes, as these would be likely sources of hybridization which could affect genetic patterns in our study (see *Hybridization assessment*, below, for further treatment of this issue).

The fifty-five headwater streams selected for sampling varied over a range of conditions needed to test our hypotheses, including size (watershed areas 193-8622 ha), disturbance histories (undisturbed, burned, and burned and disturbed), and isolation (with or without culvert barriers). In stream selection, we focused on creating a balanced sampling design that ensured both disturbance level and isolation level were well-represented over all ranges of conditions for this study. We sampled tributaries without a recent (<100 y) history of wildfire (hereafter, "undisturbed") and tributaries that had been burned throughout most of their watersheds within the last decade (1998-2003): "burned" streams had experienced stand-replacing wildfire throughout most of their watershed, and "burned and reorganized" streams had experienced stand-replacing wildfire followed by massive channel reorganization resulting from debris flows or severe floods that likely caused complete extirpation of trout throughout the length of stream channel affected.

For all three levels of disturbance (undisturbed, burned, and burned and reorganized) we sampled two levels of isolation: 'connected' or 'isolated' due to culverts. Streams considered 'isolated' had culverts that were clear barriers to adult rainbow trout movement (Clarkin et al. 2005).

Within each stream, rainbow trout were sampled via electrofishing (12B electrofisher, Smith Root, Vancouver, WA). Sampling consisted of a single up-stream electrofishing pass beginning either above a culvert or at least 300 m above of the confluence of the tributary with the Boise or Payette rivers. Sampling continued upstream until a sufficient number of rainbow trout were collected for analysis (30-35 individuals). Field crews took care not to sample family groups (see Hansen *et al.* 1997) by avoiding young-of-the-year and age class 1 fish, as well as by spreading shocking effort over a large length of the stream. After capture, rainbow trout were anesthetized with MS222 and small fin clips were taken from the caudal fin and immediately stored in 95% ethanol for later analysis.

General laboratory procedures

Total genomic DNA was extracted using DNeasy extraction kits (Qiagen Inc, Valencia, CA, U.S.A.), and was diluted to 5 ng/μl following quantification with fluorometry. Polymerase chain reactions (PCRs) and fragment sizing using an Applied Biosystems (Foster City, CA, U.S.A) Prism 3730 DNA Analyzer were performed by the Nevada Genomics Center (http://www.ag.unr.edu/genomics/, Reno, NV). We used fourteen fluorescently labeled tri- and tetra-nucleotide microsatellite loci (Table 1) isolated from

rainbow trout (Rexroad and Palti 2003) and Lahontan cutthroat trout (Oncorynchus

clarkii henshawi, Peacock *et al.* 2004). PCRs were performed in 15 µl reactions using

20ng DNA and reagent concentrations and thermal protocols listed in Table 1.

Individuals were genotyped manually using Genemapper v3.0 (Applied Biosystems).

Hybridization assessment

Throughout the last century, both rainbow trout and two sub-species of cutthroat trout

were introduced in the Boise River basin (pre-1980's stocking used Yellowstone

cutthroat trout, *Oncorhynchus clarkii bouvieri*; later plants used westslope cutthroat trout,

Oncorhynchus clarkii lewisi). Unfortunately, no genetic markers are available that

distinguish hatchery-origin from native rainbow trout, and because we could not

determine which individuals may have been affected by rainbow trout stocking in our

samples we designed our sampling protocol to try to avoid areas close to lakes or

reservoirs, common sources of non-native invasion (Adams *et al.* 2001). However,

markers are available that distinguish the various sub-species of cutthroat trout from

rainbow trout (Ostberg and Rodriguez 2004). Each individual was therefore assessed for

hybridization with cutthroat trout using a single PCR multiplex of seven primers (see

Table 2). Each primer amplifies a single-species product specific to either rainbow trout

or cutthroat trout in individuals of each parent species, or co-dominant heterozygous

products in hybrid individuals (Ostberg and Rodriguez 2004). This combination of seven

loci should confer high power to detect hybridization with cutthroat trout in our system.

For instance, a power analysis based on seven co-dominant markers suggests that the

probability of mistakenly categorizing a first generation back-crossed individual as a pure

parental type is 0.0078 (see Boecklen and Howard 1997). Individuals heterozygous at any of the seven loci were discarded from the data set.

Within-population genetic diversity

We assessed each sample for Hardy-Weinberg equilibrium by testing for excessive or deficient F_{IS} values at each locus using FSTAT (Goudet 2001), adjusting critical significance levels to account for simultaneous tests. Using FSTAT, we calculated several measures of within-population genetic diversity for each sample, including Nei's (1987) unbiased measure of gene diversity (H_E), the total number of alleles (A), and allelic richness (R_S). R_S is a rarified estimate of the number of alleles that is independent of the sample size (El Mousadik and Petit 1996; Petit *et al.* 1998; Leberg 2002). Results from rarefaction as implemented by FSTAT were identical to those using a newer method suggested by Kalinowski (2004), and for simplification of analyses we thus used the FSTAT method. We also calculated Garza and Williamson's (2001) M ratio, a potential indicator of genetic bottlenecks. The M ratio characterizes changes that occur after a bottleneck in the distribution of allele sizes relative to the number of alleles in a population. Empirical data from populations with documented demographic histories suggest that those that have been historically stable had M ratios above 0.82, while those with known bottlenecks had an M ratio less than 0.7 (Garza and Williamson 2001).

To examine relationships between within-population genetic diversity and hypothesized influences of habitat size, isolation, and disturbance history, we began by examining relationships among each measure or indicator of diversity. Spearman rank correlations

among H_E, A, R_S, and M ratio were examined to determine the degree of covariation or redundancy in each metric. Mean values and coefficients of variation for each measure were also examined to identify which metric showed the most overall variability and therefore contained a greater signal to explore the hypothesized influences of physical factors.

Based on our analysis of strong covariation among metrics and variability within each metric (see Results) we selected A, the total number of alleles, as the most appropriate measure for testing influences of physical factors on within-population genetic diversity. The total number of alleles per sample represented a broad range of counts, potentially modeled as a Poisson process (Agresti 2002). We considered Poisson regression initially, but lack of fit indicated by overdispersion (Allison 1999) suggested variance in excess of the Poisson distribution (results not shown). Accordingly, we considered negative binomial regression, an extension of the Poisson regression model with a parameter that accounts for the overdispersion (Allison 1999). With negative binomial regression, we modeled the total number of alleles (A) in each sample of rainbow trout as a function habitat size (stream catchment area, ha), isolation (presence of culverts that impeded fish movement, coded as 1 or 0), and disturbance history (burned, unburned, burned and reorganized, categorical).

RESULTS

Hybrid assessment

Of 1974 fish included in the original data set, eighty six cutthroat/rainbow trout hybrid individuals were identified and removed from further analyses. Hybrid individuals were spread across fifteen of the fifty-five populations. In these 15 populations, the percent of the original sample that was made up of hybrids ranged from 2.5 to 40, with five populations having < 10% hybridized individuals, and four having greater than 30% (maximum = 40%). Hybrid individuals could be further classified based on their specific genotypes as follows (see also Rubidge and Taylor 2004): individuals that were heterozygous at all loci were classified as F_1 hybrids (the product of a pure rainbow trout x pure westslope cutthroat trout mating); those that were homozygous at one or more loci for only one parent species were classified as backcrosses (the product of a hybrid x pure parental type), and those that had at least one locus that was homozygous for each parent species were classified as F_ns, or post-F_1 generation hybrids (the product of a hybrid x hybrid mating). Five individuals were classified as F_1 hybrids (found only in Trail and Trapper creeks), 78 were rainbow back-crosses, and one individual was deemed a post F_1-generation hybrid (found in Big Owl creek). Two individuals (one from Rattlesnake and one from Ucon creek) had cutthroat trout alleles and were removed from further analyses, but could not be categorized with confidence due to poor amplification. No pure cutthroat trout or cutthroat-backcrossed individuals were identified. Although only one of our hybrid loci (OMM 55, see Ostberg and Rodriguez 2004) had the ability to distinguish Yellowstone from westslope cutthroat trout alleles, no Yellowstone cutthroat trout alleles were identified in any individuals.

Within-population genetic diversity

Following removal of hybrid individuals, our final data set comprised 1888 assumedly

pure rainbow trout dispersed across 55 samples. None of the 770 F_{IS} value deviated

significantly from zero in either direction after correction from multiple tests, suggesting

that our samples were in Hardy-Weinberg equilibrium. Gene diversity (H_E) averaged

across loci ranged from 0.44-0.84, the total number of alleles per sample (A) ranged from

49-180, and allelic richness (R_S) ranged from 46.65-153.27. The M ratio ranged from

0.50-0.82, with 23 out of 55 populations having a ratio of 0.69 or less suggesting a

genetic bottleneck. Only one population had a ratio as high as 0.82, which based on

empirical data suggests demographic stability (Garza and Williamson 2001).

Measures of within-population diversity showed substantial variability among samples, as

indicated by the coefficient of variation, with M ratio showing the least variability, and A

showing the greatest variability (Table 3). Spearman rank correlations showed that all metrics

were highly correlated ($P < 0.0001$), but that M ratio was less strongly associated with other

metrics (Table 4). The negative binomial regression investigating the relationship between the

total number of alleles and habitat size, isolation, and disturbance history indicated that habitat

size and isolation had the most important influence on genetic diversity (Table 5, Figure 1) and

that disturbance history had insignificant impact (Table 5).

DISCUSSION

Most previous work investigating the impact of landscape factors on population

dynamics and genetic variation has focused on demographic processes assumed to occur

in static habitat (e.g., Hanski 1999; Gerlach and Musolf 2000; Arnaud 2003; Coulon *et al.*

2004; Funk *et al.* 2005; Coulon *et al.* 2006). Much less is known about the dynamics of

species that may be affected by, or even have evolved in concert with, a stochastic environment (Ross 2006; Wilcox *et al.* 2006). Salmonid fishes are one group of species that have evolved in a highly dynamic environment. Re-colonization following disturbance or movement into newly-accessible habitat can be rapid, and is frequently followed by population divergence and the emergence of life history variation (Quinn and Unwin 1993; Hendry *et al.* 2000; Quinn *et al.* 2001; Ramstad *et al.* 2004). The ability of salmonid fishes to express multiple and flexible life history strategies, particularly in relation to movement, is thought to be an adaptive buffering strategy that spreads risk across space and time and thus is a major factor enabling their persistence (Rieman and Dunham 2000). Indeed, previous demographic work on rainbow trout in our study system showed little effect of fire disturbance on rainbow trout distributions (Dunham *et al.* In prep), and results here suggest that the genetic diversity of this species as well is quite resilient to disturbance. Several other recent genetic studies demonstrate similar potential for salmonids to be resilient in the face of a highly dynamic and altered aquatic environment (e.g.,Consuegra *et al.* 2005; Neville *et al.* 2006).

However, it is likely that landscape characteristics provide a critical context in which variable movement strategies and genetic diversity may or may not be maintained, and this context may ultimately dictate the level of resiliency a species displays in response to disturbance (Dunham *et al.* 2003; Rieman *et al.* 2003; Burton 2005). Along these lines, our investigation of genetic diversity as one indicator of the potential negative effects of disturbance suggests an interesting contrast between the effects of natural and human disturbances across the landscape on rainbow trout. Despite little impact of even

catastrophic fire disturbance on genetic diversity in our study system, we found strong

evidence that isolation due to culvert barriers can cause relatively rapid (i.e., within a

century) loss of genetic variation. Isolation likely prevents fish from accessing important

refugia during a disturbance event such as a fire (Rieman and Clayton 1997), makes re-

colonization of extirpated habitats afterwards impossible, and prevents the future influx

of migrants and therefore genes. It has been suggested previously that habitat

fragmentation may have more severe effects in dendritic systems where organisms are

constrained to linear dispersal patterns (Fagan 2002), and strong negative impacts to

genetic diversity related to loss of connectivity are beginning to be documented in

salmonid fishes (Wofford *et al.* 2005; Neville *et al.* In press-a; Neville *et al.* In press-b).

In addition to the importance of habitat connectivity, our results also demonstrate that

habitat area effects influence levels of genetic diversity, consistent with growing "habitat

geometry" paradigm in metapopulation/landscape ecology (Hanski 1999; Hanski and

Gagiotti 2004).

Finally, though our study was not designed to investigate patterns of hybridization or

factors associated with them, we did find rainbow-cutthroat trout hybrids in over 25% of

our sampled rainbow trout populations. In the western United States, most studies of

trout hybridization have focused on introgression between native cutthroat trout and

introduced rainbow trout (e.g., Hitt et al. 2003; Peacock and Kirchoff 2004; Rubidge and

Taylor 2004, 2005), while less attention has been paid to the conservation of rainbow

trout populations in their native range. Interestingly, despite the long history of non-

native trout introductions in these watersheds, the large majority of our samples

contained no hybrid individuals, none of our samples were characterized as hybrid swarms, and no pure cutthroat trout or even cutthroat trout backcrosses were observed. Wile the conservation of hybridized populations remains contentious (Allendorf et al. 2004, 2005; Campton and Kaeding 2005), the characterization of many sampled populations as 'pure' rainbow trout suggests that the genetic integrity of many of these populations may still be conserved. Thus, while we caution that hybridization may be a previously unrecognized conservation concern for native rainbow trout in this region, control may still be possible if the source of hybridization is disrupted.

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Table 1. Literature references and PCR laboratory protocols for microsatellite loci used to genotype rainbow trout in the Boise River Basin, Idaho. Several sets of loci could be combined in separate multiplex PCRs (Multiplexes 1-3) as indicated in column 1.

PCR	Locus	Reference	Primer [ ] uM	PCR mix	Thermal protocol
Multiplex 1	OMM1286	Rexroad and Palti 2003	0.15	Qiagen MP †	
	OMM1295	"	0.05	"	95º for 15 min; 34 cycles of 95º (30 sec), 56º (1.5 min) and 72º (30 sec); 30 min at 62º
	OMM1178	"	0.1	"	
Multiplex 2	OCH20	Peacock et al unpublished	0.1	Qiagen MP †	
	OMM1220	Rexroad and Palti 2003	0.04	"	
	OMM1235	"	0.1	"	95º for 15 min; 34 cycles of 95º (30 sec), 62º (1.5 min) and 72º (30 sec); 30 min at 62º
	OMM1236	"	0.2	"	
	OMM1231	"	0.1	"	
Multiplex 3	OCH6	Peacock et al 2004	0.18	Qiagen MP †	95º for 15 min; 25 cycles of 95º (30 sec), 67º-52º touchdown (1.5 min) and 72º (30
	OCH9	"	0.06	"	sec);10 cycles of 95º (30 sec), 54º (1.5 min) and 72º (30 sec); 30 min at 62º
	OCH10	"	0.12	"	
Single locus	OMM1234	Rexroad and Palti 2003	0.2	Single §	95º for 5 min; 36 cycles of 95º (30 sec), 66º (30 sec) and 72º (30 sec); 30 min at 72º
Single locus	OMM1177	"	0.2	"	95º for 5 min; 36 cycles of 95º (30 sec), 58º (30 sec) and 72º (30 sec); 30 min at 72º
Single locus	OMM1173	"	0.2	"	95º for 5 min; 36 cycles of 95º (30 sec), 67º (30 sec) and 72º (30 sec); 30 min at 72º
Single locus	OMM1272	"	0.2	"	95º for 5 min; 36 cycles of 95º (30 sec), 67º (30 sec) and 72º (30 sec); 30 min at 72º

† Qiagen Multiplex mix (commercial) with 1 unit HotStart DNA Polymerase, 3 mM MgCl2 at pH 8.7

§ Single-locus PCR with 1X buffer with 3.5 mM MgCl2, 0.83 mM dNTPs, 1 unit Titanium taq

Table 2. Literature reference and PCR laboratory protocols for multiplexed loci used to identify rainbow-cutthroat trout hybrids in the Boise River Basin, Idaho.

PCR	Locus	Reference	Primer [ ] uM	PCR mix	Thermal protocol
Hybrid multiplex	OMM55	Ostberg and Rodriguez 2004	0.2	Qiagen MP †	
	OCC38	"	0.2	"	
	OCC37	"	0.2	"	
	OCC34	"	0.2	"	$95°$ for 15 min; 34 cycles of $95°$ (30 sec), $57°$ (1.5 min) and $72°$ (30 sec); 30 min at $62°$
	OCC42	"	0.2	"	
	OCC35	"	0.1	"	
	OCC36	"	0.4	"	

† Qiagen Multiplex mix (commercial) with 1 unit HotStart DNA Polymerase, 3 mM MgCl2 at pH 8.7

Table 3. Descriptive statistics of genetic diversity measurements of rainbow trout populations in the Boise River basin

Variable	Mean value	Minimum value	Maximum value	Coefficient of variation
H_E	0.74	0.44	0.85	10.41
R_S	108.7	46.65	153.27	21.57
A	126.98	49	180	23.75
M ratio	0.7	0.5	0.82	9.11

Table 4. Spearman rank correlation coefficients for genetic diversity measurements of rainbow trout populations in the Boise River basin. R_S = allelic richness, A = total number of alleles.

	R_S	A	M ratio
H_E	0.88	0.87	0.63
R_S		0.98	0.55
A			0.57

Table 5. Likelihood ratio estimates and associated statistics for negative binomial regression relating the total number of alleles to various landscape parameters. TRT B,BR,UN = Treatment: Burned, Burned and reorganized, and Undisturbed

Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr>ChiSq
Intercept	1	4.6022	0.0641	4.4744	4.7306	5148.28	<0.0001
Habitat size_ha	1	0.0001	0.0000	0.0000	0.0001	12.13	0.0005
Culvert N	1	0.1835	0.0591	0.0653	0.3013	9.63	0.0019
Culvert Y	0	0.0000	0.0000	0.0000	0.0000	.	.
TRT B	1	0.0272	0.0667	-0.1060	0.1604	0.17	0.6834
TRT BR	1	0.0320	0.0758	-0.1188	0.1840	0.18	0.6724
TRT UN	0	0.0000	0.0000	0.0000	0.0000	.	.

Figure 1. Total number of alleles versus catchment basin area in hectares for sites connected and without culverts (green) and isolated by culverts (red) in the Boise and Payette river basins.